

The Action of Mitomycin C
on the Bristle-Forming Apparatus
of *Phormia regina*

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The Action of Mitomycin C on the Bristle-Forming Apparatus of *Phormia regina*

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Summary. Mitomycin C, a known inhibitor of DNA synthesis, was injected into white prepupae of *Phormia regina*. Adults which developed from these prepupae showed alterations of the bristle pattern, loss of whole bristle organs, and the formation of bristles without sockets or sockets without bristle shafts. Dose-dependence was found for all modifications. For the abdominal microchaetae, the period of maximum sensitivity to the drug began at 16 h after puparium formation, that is well after all of the macrochaetae and most of the microchaetae of the thorax and the head had grown insensitive. Bristle forming trichogen and tormogen cells developed high degrees of polyteny with distinctly banded chromosomes. Photometric determination of the amount of Feulgen-DNA per nucleus led to estimations of DNA classes ranging from 256 C to 2048 C. DNA contents of nuclei from Mitomycin C treated animals were significantly lower during the actual growth of the bristle apparatus, but reached approximately the same level as the controls prior to the time of emergence. Cytological investigations proved that doses of Mitomycin C which yielded bristle organs either without sockets or without shafts do not affect the differential division of the bristle mother cell. Polytene chromosomes damaged by Mitomycin C displayed a diffuse and irregular banding pattern. Possible modes of action of Mitomycin C on replicating polytene chromosomes are discussed.

The epidermis of insects represents a particularly convenient system for the study of processes of cell determination and differentiation. Epidermal cells are arranged in a monolayered epithelium, and the spatially well defined bristle pattern allows the easy identification of precursor cells for specific bristle organs. These originate from a bristle mother-cell by differential mitosis. The smaller tormogen daughter-cell produces the socket, whereas the bristle shaft is synthesized by the larger trichogen cell (Robertson, 1936; Lees and Waddington, 1942). The nuclei of these cells reach high degrees of polyteny during pupal development (Ribbert, 1967). The application of the antibiotic Mitomycin C (MC) to *Drosophila melanogaster* results in the formation of bristles without sockets among other integumental modifications (Tobler, 1969; Tobler and Pfluger, 1969; Tobler and Burckhardt, 1971). These defects might be due to an inhibition of the differential cell division after MC treatment, or, alternatively, to a repression of polytenization.

This paper presents data from cytological and cytophotometric investigations on this problem. *Phormia regina* was chosen for these experiments, because the larger cell size permits a more detailed analysis of the effect of the drug on cell morphology and DNA content. Preliminary experiments with MC on larvae or pupae of *P. regina* led to similar modifications on the bristle pattern as have been described for *D. melanogaster*.

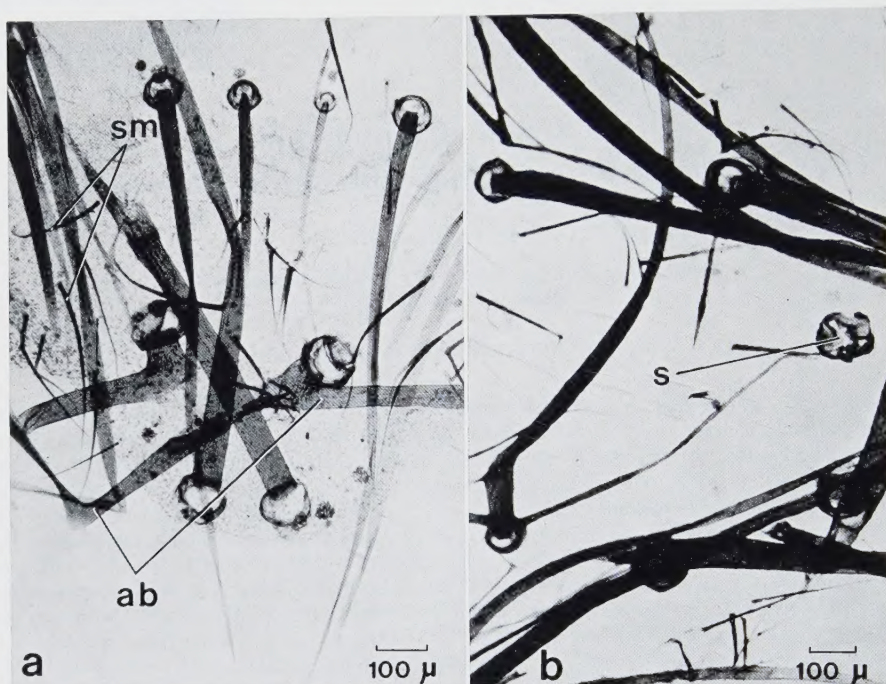


Fig. 1 a and b. Modifications of the bristle organs after injection of MC ($10 \mu\text{g/g}$) into pupae of *P. regina*. (a) Scutellar and posterior mesothoracic region with socketless microbristles (*sm*) and aberrant bristle shafts (*ab*); (b) Socket of a macrobristle without bristle shaft (*s*) on the scutellum

Material and Methods

1. Rearing conditions

The flies were an inbred laboratory strain, kept at 25°C and raised on a sugar diet which was supplemented after 1 week with fresh horse liver. Immobile prepupae formed $6\frac{1}{2}$ –7 days after egg deposition. Their physiological age is given relative to the formation of the white prepuparium stage.

2. Application of MC

MC dissolved in sterile Ringer solution ($0.8 \mu\text{g/ml}$) was injected by the method of Baumann (1969) into the 4th abdominal segment of white prepupae, except where otherwise stated. The cuticle had been pierced laterally using a sharp needle. To determine the most effective dose of MC on the formation of aberrant bristle types, different amounts of the solution were tested. The dose used for the photometric measurement series and for cytological investigations was $10 \mu\text{g/g}$ live weight. This resulted in a pupal mortality of 30% with most imagoes being unable to release themselves from the pupal case. Control animals received Ringer solution alone.

3. DNA Staining and Isolation of Single Bristle-Forming Cells

After the pupae had been freed from the pupal case, the dorsal thorax was dissected using iridectomy scissors, and the adhering muscle and fat tissue was removed. The cuticle with underlying epidermis was then fixed in ethanol-acetic acid (3:1) for 2 h. Hydrolysis

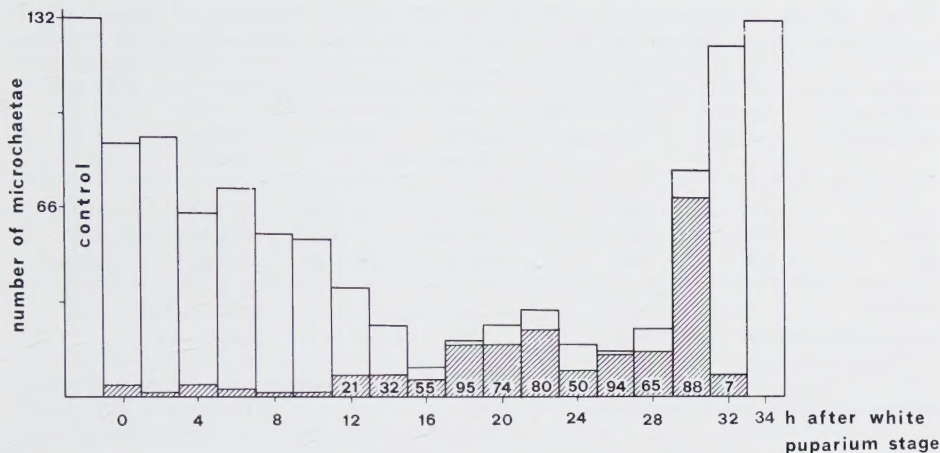


Fig. 2. The effect of MC injection into pupae of different ages on the number of differentiated abdominal microchaetae and on the formation of microchaetae without sockets in % of differentiated bristle anlagen (see figures in hatched part of the columns). The area counted was 0.4 mm^2 on the second abdominal tergite. Injected dose of MC: $5 \mu\text{g/g}$

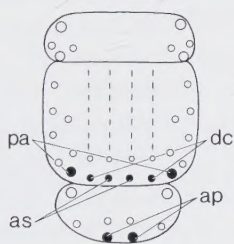


Fig. 3. Schematic drawing of the pattern of posterior thoracal and scutellar macrochaetae of *P. regina*. The following bristles (•) have been analyzed: *ap* apical, *pa* postalar, *dc* posterior dorsocentral, *as* posterior acrostichal

was carried out at room temperature in 5 N HCl for 30 min. The nuclei were stained with Schiff's reagent (Feulgen dye) according to the method of Deitch (1966). Some of the epidermal tissues so treated were examined to determine the growth parameters of the apical bristle shaft (*ap*; cf. Fig. 3) and of the nuclei of specific bristle-forming cells during pupal development.

For photometric determination of the DNA content, the polytene nuclei were separated with fine tungsten needles in a drop of glycerol. A constant thickness of the specimens ($20 \mu\text{m}$) was obtained by placing strips of aluminium foil under the coverslip. The fading of the dye could be prevented for up to 2 weeks by sealing the preparations with nail polish and storing them in the dark at 4°C .

4. Preparation of Flies for Microscopic Inspection

For the analysis of the bristle patterns on the abdomen, the tergites of the metamorphosed flies were dissected from 5 day old pupae, and the first four dorsal abdominal segments were separated from the rest of the abdomen. The tergites were then fixed in ethanol-acetic acid (3:1) for 30 min. Care was taken not to loosen any bristle when adhering tissue was removed. After washing in water the tergites were embedded in Faure's solution.

Table 1. Effects of different doses of injected MC on the frequency of loss of whole macrochaetal organs, sockets and bristle shafts on the thorax and scutellum of *P. regina*

Injected dose of MC ($\mu\text{g/g}$ live weight)	Number of treated pupae	Number of macrochaetae examined	Number of missing structures (%)			X^2 -test ^a <i>p</i>
			bristle organs	sockets	bristle shafts	
1.7	31	868	10 (1,2)	2 (0,2)	7 (0,8)	> 0.05
3.5	32	896	53 (5,9)	17 (1,9)	16 (1,8)	> 0.5
10	20	560	119 (21,3)	49 (8,8)	13 (2,3)	< 0.001
Control:						
Ringer solution	10	280	0	0	0	

^a The X^2 -test is applied to compare the number of missing sockets with the corresponding number of missing bristle shafts

5. Measurement of Feulgen-DNA Content of Nuclei

Photometric determinations were performed with a Zeiss microscope equipped with a Xenon XBO 150 lamp, monochromator, $0.5\ \mu\text{m}$ scanning, objective 40/0.75 and modular electronics with a classification unit for the integral of extinction. The integral of absorption was calculated at 570 nm. The systematic error was 5% since the large flattened nuclei had to be scanned with a suboptimal objective.

Results

1. The Action of MC on the Differentiation of the Bristle Organ

After injection of MC into pupae of *P. regina*, the following modifications were observed: (i) loss of whole bristle organs, (ii) formation of socketless microbristles and, to a lesser extent, of socketless macrobristles, (iii) differentiation of aberrant bristle shafts (Fig. 1a, b). These results confirm the earlier findings of Tobler and co-workers (Tobler, 1969; Tobler and Pfluger, 1969; Tobler and Burckhardt, 1971) on *D. melanogaster*. In addition, the following observation proved to be new: After treatment with MC, *P. regina* developed not only bristles without sockets, but also sockets without bristle shafts (Fig. 1b). When low doses ($1.7\ \mu\text{g/g}$) of MC were applied, sockets without bristle shafts were even more frequent than bristles without sockets, although this difference was statistically insignificant (X^2 -test, Table 1). Dose dependence was found for all modifications recorded. We therefore conclude that MC was the agent producing those alterations.

As in *D. melanogaster* (Tobler and Burckhardt, 1971), the bristle-forming cells of abdominal microchaetae were almost insensitive to the drug injected into prepupae or young pupae. For *D. melanogaster*, Garcia-Bellido and Merriam (1971) and Guerra et al. (1973) showed that the abdominal histoblasts—in contrast to all other imaginal discs—remain quiescent throughout larval development and start cell division only after puparium—formation. Differential cell divisions of the microchaetal mother-cells in abdominal sternites and tergites take place 32 h after puparium formation. A similar developmental schedule has been suggested for other flies (Kowalewsky, 1887).

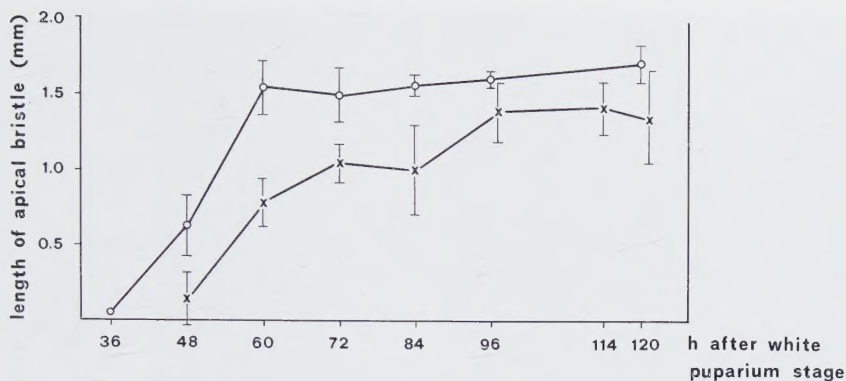


Fig. 4. The growth of the shaft of the ap-bristle in control (○—○) and MC-treated pupae (×—×) during pupal development. Standard deviations (in % of corresponding mean length) are indicated as vertical lines. Injected dose of MC: 10 μ g/g

In order to determine the sensitive phase for abdominal microchaetal modifications of *P. regina*, MC was injected into pupae of different ages. The results are summarized in Figure 2. MC-injections resulted in a significant reduction of whole bristle organs, with a maximum effect at 16 h when about 90% of the microchaetae are not formed. The sensitive phase for the formation of microbristles without sockets follows, between 16 h and 30 h, when 50% or more of the bristle organs formed were socketless. However, no distinct sensitive phase could be established for the formation of sockets without bristle shafts; they are found at a very low frequency of about 1.5% throughout the time span covered by this experiment.

2. The Effect of MC-Treatment on Bristle-Growth

For a comparison of the process of bristle-growth in control and MC-treated cells, the development of the apical bristle (ap; cf. Fig. 3) was studied. This bristle was chosen because it develops first and reaches the greatest size. The length of the secreted shafts was measured at 12 h intervals. The results are shown graphically in Figure 4. The first stage of development could be detected in the controls at 36 h by the secretion of macrochaetal papillae. No such outgrowth could be recognized in MC-treated trichogen cells at this time. They appear at 48 h only. It was not possible to decide whether this delay was due to a slower growth-rate of treated cells or rather to a delayed initiation of growth of the bristle-shaft. Bristle length was reduced by some 30% to 40% during the extensive growth period and was approximately 20% shorter at the end of pupal life.

3. Cytological Parameters of Control and MC-Treated Nuclei of the Bristle-Forming Apparatus

The trichogen and tormogen nuclei possess six giant polytene chromosomes with a distinct banding pattern (see Fig. 5b). The trichogen nuclei of apical (ap, cf. Fig. 3) and postalar bristles (pa, cf. Fig. 3) are about 4 times larger in volume

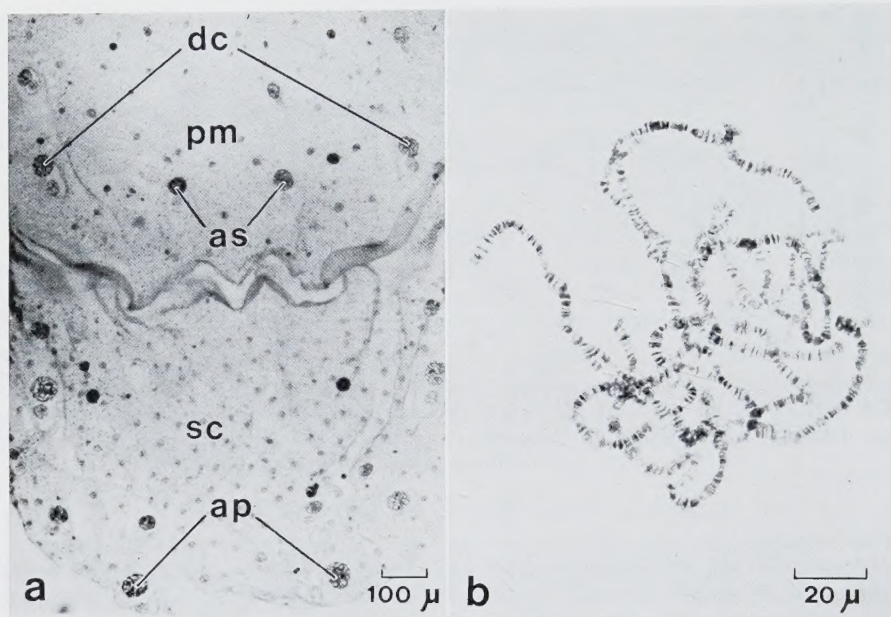


Fig. 5a and b. Polytene nuclei of bristle-forming cells at 48 h after white puparium stage (Feulgen staining). (a) Posterior mesothoracal (*pm*) and scutellar (*sc*) region with apical (*ap*), dorsocentral (*dc*) and acrostichal (*as*) bristle cells; (b) Trichogen chromosomes of the *ap* bristle cell

than the corresponding tormogen nuclei and attain a higher degree of polyteny. The ratio of nuclear volumes is about 2:1 in dorsocentral and acrostichal forming cells, respectively (see Fig. 5a). A polytene chromosome structure could also be established in all microbristle cells of the thorax, the abdomen and the legs. 18 h after puparium formation, the first pairs of enlarged cells which will give rise to the long *ap* bristles on the scutellum could be recognized. The diameter of the trichogen nuclei was about $20\ \mu$ at the 24 h stage. It reached its maximum size of about $70\ \mu$ between 72 and 84 h. This rapid increase in size was paralleled by extensive endoreplication of the chromosomes (cf. Section 4, p. 152). Slow gradual reduction of the nuclear volume and first signs of degradation of chromatin were evident at the stage of 96 h when the fully formed bristles became amber. Near the time of emergence, pycnosis of all bristle-forming cells was obvious: the chromosomes were difficult to spread and the uptake of Feulgen dye was reduced, apparently owing to DNA-decomposition.

In contrast to control pupae injected with Ringer solution, all MC-treated, animals showed the following signs of retarded development: (i) retarded evagination of head, wing and leg primordia, (ii) delayed degradation of polytene cells of the larval fat body and the epidermis, (iii) delayed growth of bristle-forming cells. All these deviations from normal development were subjected to considerable individual variation. Bristle-forming cells could only be distinguished 4 to 6 h later than in controls. Eye and bristle pigmentation was deferred for

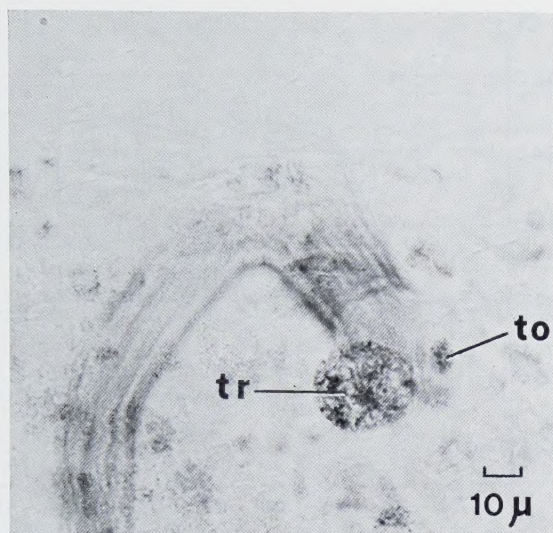


Fig. 6. Formation of a bristle shaft without socket after injection of MC ($10 \mu\text{g/g}$) into a pupa of *P. regina*. The trichogen (*tr*) and the pycnotic tormogen nucleus (*to*) are located at the base of the bristle shaft

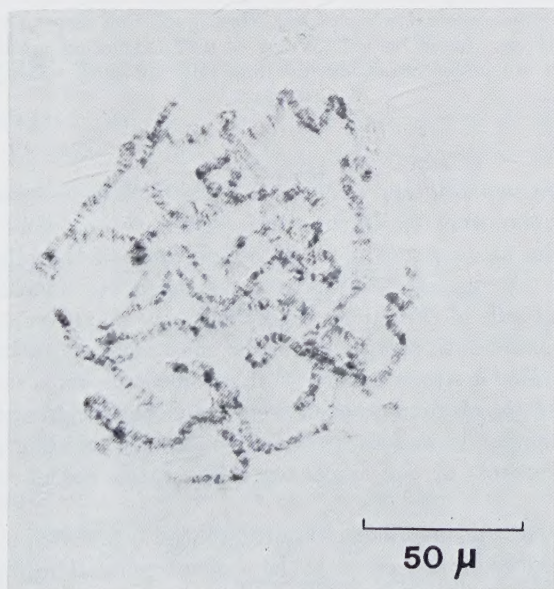


Fig. 7. Polytene chromosomes of a trichogen ap-bristle cell at 48 h after injection of MC ($10 \mu\text{g/g}$) into a pupa of *P. regina*. The chromosomes show irregular banding pattern and faint staining of the bands

8 to 12 h. Towards the end of pupal life, delays in pupal development of 18 h or more were noted.

After appearance of bristle pigmentation (around 84 h), the abnormal bristles could be recognized by their morphology. All of these future socketless bristles,

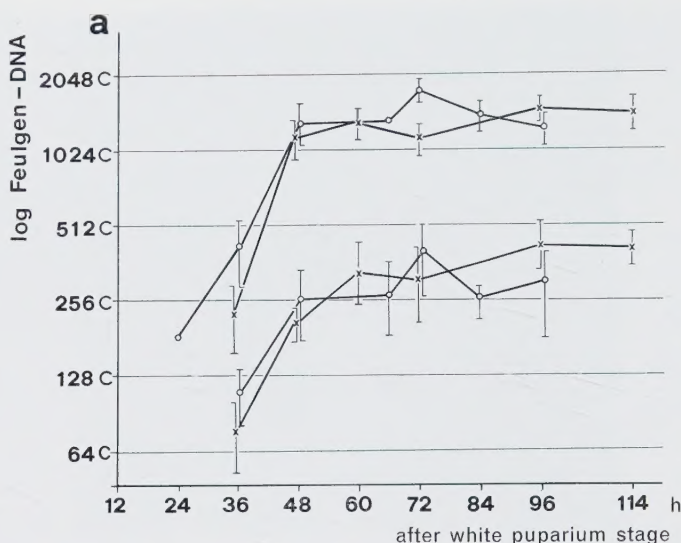


Fig. 8a—c. The increase of Feulgen-DNA amount during pupal development in control (○—○) and MC-treated (×—×) animals: (a) apical, (b) postalar, (c) posterior dorsocentral and acrostichal bristle forming cells. Upper curves, trichogen nuclei; lower curves, tormogen nuclei. The ages of pupae are given in h after white puparium stage. On the ordinate are given classes of polyteny (based on a 2C-value of 8.67 extinction units; see Discussion). Standard deviations are indicated as vertical lines. MC (10 µg/g) was injected into white prepupae

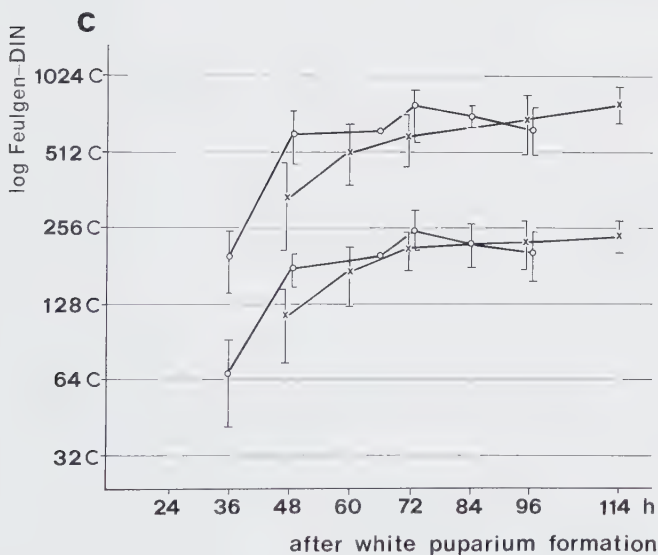
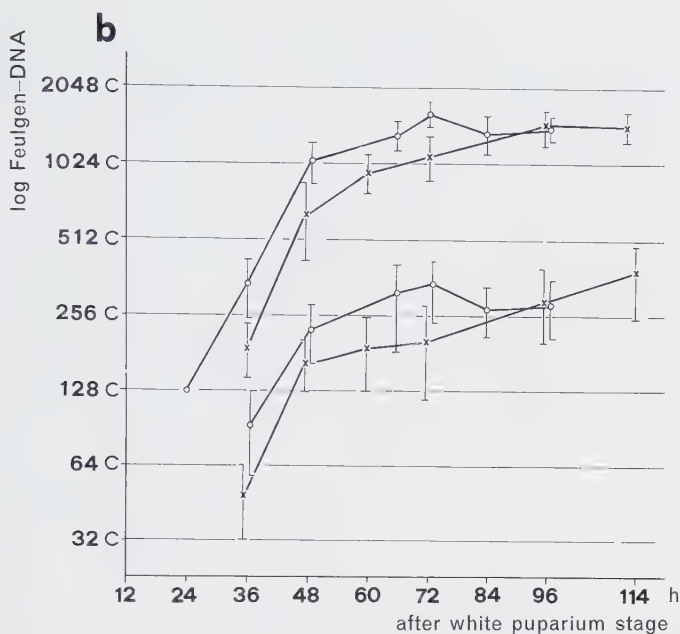
however, possessed two polytene nuclei, one—the trichogen—which secreted the bristle shaft, and the other which, due to its DNA content and position, could be identified as the tormogen (Fig. 6). Although most of the effect of MC is on microchaetae, our cytological analysis was restricted to macrochaetae because the bristle-forming cells of the microchaetae were too small for an investigation.

Microscopical examination of polytene chromosomes replicating under the influence of MC revealed a strong tendency of chromosome arms to adhere to each other. Furthermore, the chromosomes showed many constricted and swollen zones, faint staining of the bands, and an irregularly organized banding pattern (Fig. 7). Compared with controls of the same age, MC-treated nuclei were also smaller in volume.

In conclusion, MC did not block the differential cell division when aberrant bristles were produced, but seemed to have some general inhibitory effect on DNA synthesis and polytenization.

4. Photometric Determination of Feulgen-DNA Content of Control and MC-Treated Trichogen and Tormogen Nuclei

a) *Increase of Nuclear DNA Content during Pupal Development.* During pupal development, the trichogen and tormogen cells of ap, pa and dc/as bristles (cf. Fig. 3) were isolated at 12 h intervals and relative Feulgen-DNA contents of their nuclei were determined photometrically, for both control and MC-treated pupae. Isolation of bristle-forming nuclei before 36 h after puparium formation



was not possible since the nuclei were still too small. Control nuclei of the 60 h stage had to be prepared six hours later, because morphogenetic movements made it impossible to attribute a polytene cell pair with certainty to one particular bristle organ. This was not true for MC-treated animals since the morphogenetic movements in these pupae were delayed. The DNA content of 12 to 36 cell pairs was measured for every stage and bristle type. Means and standard deviations for both experimental series are depicted in Figure 8.

Table 2. Log Feulgen-DNA extinction values of control and MC-treated nuclei of the 36 h and 72 h series

Type of nucleus		Stage	Untreated controls		MC treated		U-test
		h	$\bar{x}$	s	$\bar{x}$	s	p
ap	tr	36	183	49	97	29	< 0.01
	to	36	46	12	32	10	< 0.01
	tr	72	767	79	497	71	< 0.01
	to	72	173	59	138	46	< 0.05
pa	tr	36	145	39	82	20	< 0.01
	to	36	40	15	21	7	< 0.01
	tr	72	706	80	483	98	< 0.01
	to	72	145	42	87	35	< 0.01
dc/as	tr	72	348	53	260	63	< 0.01
	to	72	111	20	93	16	< 0.01

Abbreviations: ap = apical, pa = postalar, dc = dorsocentral, as = acrostichal, tr = trichogen nucleus, to = tormogen nucleus, $\bar{x}$ = mean, s = standard deviation, p = probability.

All curves indicate an increase in the amount of Feulgen-DNA per nucleus during pupal development. In control nuclei, the maximum amount of Feulgen-DNA is reached at 72 h after puparium formation. A slight decrease down to 80% of the maximum values on the following day is typical. Under the influence of MC, synthesis of nuclear DNA in all bristle cells is slowed down and prolonged until the time of emergence. DNA values of MC-treated nuclei do eventually reach the same maximum level as controls, but about 24 to 48 h later. The range of variation of single extinction values in MC-treated series is about 5 to 10% wider than that of the control series. This is probably caused by some nuclei which lag behind controls of the same age by one or even two rounds of replication.

b) Statistical Tests. For statistical comparison of the Feulgen-DNA contents of control and MC-treated nuclei, the values of two developmentally meaningful stages were selected: The 36 h stage, when the first signs of bristle shaft growth were observed and the 72 h stage when the maximum amount of Feulgen-DNA in control nuclei was scored. The parameter free U-test after Wilcoxon, Mann and Whitney (Sachs, 1974) was applied to all bristle types. Table 2 shows that the content of Feulgen-DNA in MC-treated bristle-forming nuclei is significantly lower than in untreated controls at the stages mentioned. Data for 36 h stage dc/as nuclei are missing since the MC-treated cells of this stage were still too small to be isolated for photometric measurements. On average, the mean extinction values of MC-series attain 64% of the trichogen, and 70% of the tormogen control values, respectively.

Discussion

1. DNA-Amounts and Estimation of Classes of Polyteny

The DNA content of nuclei determined as extinction values was expressed in terms of classes of polyteny, which are based on a 2C value obtained by

measuring the Feulgen-DNA content of diploid epidermal cells. 43 small cells were scanned with the same objective as the giant nuclei. This resulted in a relatively high variability of the computed 2 C to 4 C values, as would be expected in an actively dividing tissue. Based on the Feulgen-DNA estimates for diploid epidermal cells (8.67 ± 2.12 extinction units), the extent of polyteny for trichogen nuclei of the apical bristles would fit projected means of 2^{10} n to 2^{11} n (1024 C to 2048 C).

A verification of this estimation was attempted by comparing the observed average nuclear diameters of diploid epidermal ($\approx 7 \mu$) and fully grown trichogen cells of apical bristle organs. The maximum diameter of these nuclei is about 70μ (see p. 150). This tenfold increase in diameter would correspond to a thousandfold increase in volume. Assuming that nuclear volume is proportional to the DNA-size-class (Ribbert, 1967), this would confirm the estimated degree of polyteny of 2048 C. The maximum diameter of dorsocentral and acrostichal trichogen nuclei was found to reach 55 to 60μ , which fits well with the calculated DNA-class of 1024 C.

2. The Effects of MC on the Differentiative Cell Division and on Polytenization

The cytostatic action of MC is due to a crosslinking of DNA chains by covalent bonding of G-C sites which prevents complete separation of complementary strands during replication (Szybalsky and Iyer, 1964). MC is lethal for cells in actively dividing tissues, but apparently not for replicating but non-dividing polytene cells. This is understandable in that polytene chromosomes contain multiple copies of most genetic loci. Thus, any irreversibly MC-linked locus on a chromonema would have several functional equivalents laterally arranged.

It is not known when the differential division of bristle mother-cells occurs. We have shown that bristles without sockets are always associated with two polytene nuclei. Thus, MC has no effect on the differential division, or more likely, this event took place before the application of the drug. MC however did have a marked effect on the rate of polytenization and we think this is a more likely cause for these incomplete bristle organs. The dose dependent loss of whole bristle organs on the other hand could be due either to a blockage of the differential mitosis, or, alternatively, to the concomitant inhibition of both daughter cells. Unfortunately, no experimental data are available to clarify this point.

The effect of MC on the nuclei of developing bristle-forming cells has a qualitative and a quantitative aspect. The binding of MC might also inhibit the ultimate transcription of the affected genes. Some of these may be necessary for normal trichogen or tormogen formation and thus the defect may to some extent be due to the inactivity of these genes. This qualitative aspect, coupled with the quantitative inhibition of polytenization might determine a threshold level which decides the survival or death of a damaged polytene cell.

The decreasing number of complete microbristles formed on abdominal tergites when MC is injected between 0 and 16 h after puparium formation (cf. Fig. 2) can be interpreted as a blockage of mitotic cell divisions during the proliferative growth period of the histoblasts. During the sensitive period for the

formation of abdominal microchaetae without sockets (16 h to 30 h) the number of bristles is closer to normal. However, most of them now lack a socket. In this period, MC might be affecting both differential mitosis and polytenization of the generated trichogen and tormogen cells. Krishnakumaran et al. (1967) have reported similar investigations on DNA synthesis during metamorphosis of the moth *Antheraea polyphemus*. When pupae were injected with a single dose of MC within 20 h after pupariation, developing adults had virtually no scales. The authors concluded that the antibiotic had blocked differential mitosis in the epidermis. However, we believe that MC could well have inhibited the process of polyploidization which normally follows in these scale-forming cells. In contrast to an endoreplication by polytenization where the newly formed DNA strands remain laterally arranged to the preexisting chromosome, MC induced cross-links could very effectively prevent the complete separation of the synthesized daughter chromosomes, necessary for each step of polyploidization.

3. Delayed Polytenization in Tormogen and Trichogen Cells after MC-Treatment

Our results show that polytenization in MC-treated bristle-forming cells is significantly retarded during the actual growth period of the bristle shaft, but DNA synthesis continued and reached approximately the same levels as in controls by the end of pupal development (cf. Fig. 8). This surprising result is possibly merely a methodological artifact: at the early stages of development (36 h to 60 h), it was possible to isolate nearly all bristle-forming cells of the investigated bristle types to determine the DNA content of their nuclei. At later stages, owing to the progressing pycnosis of the chromatin, we rarely succeeded in recognizing and dissecting the bristle precursor cells with heavily damaged nuclei. Therefore the determination of the mean DNA amount per treated series at later stages (72 h to 114 h) is perhaps only representative of the slightly damaged nuclei, which had been able to correct the lesion by repair synthesis. Thus, these cells would have secreted a fully-formed bristle organ with a somewhat shorter bristle shaft. There is a positive correlation between the lower degree of polyteny and the diminished biosynthetic potency of MC-treated trichogen cells (compare Fig. 4 and Fig. 8). This argues against the suggestion that the delay in polytenization is the result of an unspecific retardation in development, since bristle shaft secretion in MC-treated cells starts and continues with significantly reduced DNA contents compared to controls.

4. Differential Sensitivity of Bristle-Forming Cell Types Towards MC

Microchaetal primordia proved to be much more sensitive to the drug, as expressed by the loss of bristle shafts or sockets, than the respective macrochaetal primordia (compare Fig. 2 and Table 1). This trend is also manifested among the macrochaetae themselves, since the small dorsocentral and acrostichal macrochaetae show occasional loss of bristle shafts or sockets, whereas the large apical bristle organs are never affected. Tormogen cells are much more sensitive

to MC than the larger trichogen cells (Table 1, Conc. 10 $\mu\text{g/g}$ live weight). In *D. melanogaster*, similar doses of MC resulted in high frequencies of socketless micro- and macrochaetae, whereas sockets alone could never be recognized with certainty (Tobler, 1969; Tobler and Burekhardt, 1971). It could be argued that in our experiments all sockets without bristle shafts are artifacts of preparation. However, their typically irregular shape as well as the deposition of cuticle at the normal insertion site of the bristle shaft (cf. Fig. 1 b) make this interpretation very unlikely.

We interpret the indication that smaller bristle-forming cells are more susceptible towards the detrimental action of MC than larger ones, as follows: at the time of MC injection (white puparium stage ± 1 h), all macrochaetal and many microchaetal mother-cells have passed differential mitosis and their daughter cells are engaged in DNA synthesis. The rate of replication and consequently the extent of polyteny of different bristle-forming cells at application of MC depends upon their programmed final degree of polyteny. Therefore, the larger trichogen nuclei of macrochaetae would have the greatest probability that a given genetic locus will have functional equivalents. The higher sensitivity of the tormogen nucleus may be due to both delayed and slower DNA synthesis which would result in a longer exposure of the chromatin in a low C value state. The increased sensitivity of smaller cells towards MC could also be interpreted as a function of the volume/surface ratio of the nucleus. Small nuclei offer relatively larger surface areas than more voluminous ones. Consequently, the probability for diffusion of randomly dispersed MC molecules from the pupal hemolymph and cytoplasm into a given amount of nucleoplasm is increased.

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Curriculum vitae

Schaerer Hansrudolf, geboren am 16. Oktober 1940 in Frauenfeld, Bürger von Zürich. Nach Besuch der Grundschulen in Baden trat ich 1956 in das Aarg. Lehrerseminar in Wettingen ein, wo ich 1960 mit dem Lehrpatent für Primarlehrer abschloß. Nach 3 Jahren Lehrtätigkeit in Oberentfelden AG besuchte ich zunächst die „Kurse für Turnen und Sport“ an der ETH Zürich, wo ich im Herbst 1964 das Eidg. Turnlehrerdiplom I erwarb. Seit dem WS 64/65 war ich an der Phil. II-Fakultät der Universität Zürich, Hauptfach Zoologie, immatrikuliert. 1970 bestand ich die Diplomprüfung als Naturwissenschaftler.

Die vorliegende Dissertation stand unter der Leitung von Herrn Prof. Dr. Heinz Tobler, jetzt Universität Fribourg.

Während meinen Studien besuchte ich Vorlesungen und Kurse bei folgenden Damen und Herren Professoren und Dozenten: Akert, Andresen, Batschelet, Biegert, Birnstiel, Boesch, Burla, Chen, Dreiding, Eugster, Graber, Guyan, Hadorn, Hediger, Henking, Jungen-Hauschteck, Knüsel, Ludwig, Markgraf, Munz, Nöthiger, Nüesch, Rutishauser, Sauter, Schaeppi, Schlittler, Schüepp, Stüssi, Suter, Ursprung, Tardent, Töndury, Wagner, Wanner, Wehner, Weissmann, Woodtli, Ziswiler.

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